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Rabbit masseter expresses the cardiac α myosin heavy chain gene

Evidence from mRNA sequence analysis

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The presence of myosin α heavy chain in the rabbit masseter has been previously suggested at the protein level [(1991) Basic Appl. Myol. 1, 23–34; (1991) Histochem. J. 23, 160–170]. To confirm this finding, we cloned most of the mRNA corresponding to the myosin heavy chain S2 subfragment. PCR analysis and subsequent nucleotide sequence determination of the amplified cDNA demonstrates the presence of a myosin α heavy chain mRNA in rabbit masticatory muscles.

Rabbit masseter muscle; Myosin sequence; PCR; α Myosin heavy chain

1. INTRODUCTION

Skeletal muscles express several myosin isoforms, in different relative amounts, depending on muscle type, species and age (for review, see [3]). One of these isoforms, the slow-type myosin I, composed of β heavy chains and of slow-type or ventricular light chains, is also found in cardiac muscle. The other heavy chain found in the cardiac muscle, the α heavy chain, was thought to be specific of the cardiac muscle. However, several workers have recently suggested, from immunological and electrophoretic data, that the α heavy chain, or an α-like heavy chain, was also present in some skeletal muscles, such as the rabbit and man masticatory muscles [1,2,4] and the muscle spindle fibers [5].

In this paper, we present the amino acid sequence of most of the S2 subfragment of the presumptive myosin α heavy chain found in the rabbit masseter and compare it to the corresponding sequences of cardiac α and β, and skeletal fast-type heavy chains [6].

2. EXPERIMENTAL

Total RNA was extracted from the masseter, the retractor mandibulae, the atria, the soleus, the extensor digitorum longus (EDL), and the diaphragm of an adult rabbit as described by Chomczynski and Sacchi [7]. The RNA was treated with DNase I (Promega, 5 U/μg of RNA) for 15 min at 37°C. First-strand cDNA was synthesized for 60 m at 37°C from 5 μg of total RNA using Moloney Murine Leukemia Virus reverse transcriptase (Gibco BRL Life Technologies; 400 U) and a random primer hexamer (Promega; 0.5 μg). Oligonucleotide primers for the polymerase chain reaction (PCR) were 5′-GGAATTCGGGCGCATCAAAGAGTCCCTGGAGAAGTCG-3′ (the 5′ *Eco*RI restriction site plus nucleotides 1,260–1,289 from the sequence in [6]) and 5′-CGCTCGAG-CAGCTGACTGATGTCAAACTC-3′ (the 5′ *Xho*I restriction site plus nucleotides complementary to nucleotides 1,961–1,941 from the sequence in [6]). DNA was amplified with Taq polymerase (2.5 U, Bioprobe). After an initial incubation of 5 min at 92°C, the reaction was cycled 30 times for 1 min at 92°C, 2 min at 60°C and 3 min at 72°C, and terminated by incubation for 5 min at 72°C. To test for amplification of genomic DNA which could contaminate the RNA preparation, a control was included in which reverse transcriptase was omitted. The PCR products were analyzed on 12% polyacrylamide gels and visualized under UV by ethidium bromide fluorescence.

The fragments amplified from masseter and atrial RNAs were purified, digested with *Eco*RI and *Xho*I and subcloned in the corresponding sites of pB II KS (Stratagene). Double-stranded DNA was sequenced using the Sequanase 2.0 kit (United States Biochemical) and [^{35}S]dATP (Amersham), with the reverse and universal pUC primers, the primers used in the PCR reaction, and an internal primer.

3. RESULTS AND DISCUSSION

The sequences of the oligonucleotide primers used for PCR amplification were chosen from two regions of the myosin heavy chain sequence known to be different in the α, β, and fast-type myosin heavy chain isoforms [6]. Amplification of masseter and atrial cDNA led to the synthesis of a single fragment of the expected size (701 nucleotides), corresponding to most of the myosin S2 subfragment (Fig. 1A). The same result was obtained with another masticatory muscle, the retractor mandibulae (not shown). The fact that there was no amplification in the absence of reverse transcriptase demon-

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Abbreviations: PCR, polymerase chain reaction; EDL, extensor digitorum longus.

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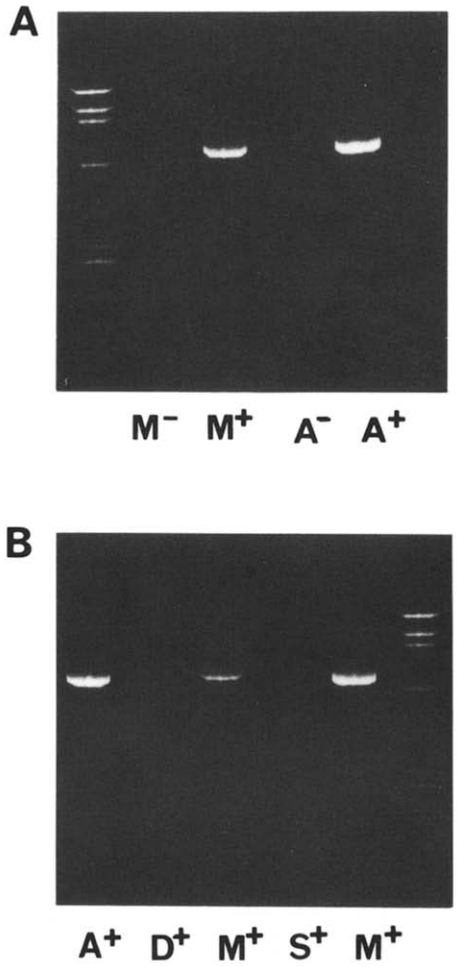

Fig. 1. PCR amplified cDNA (two preparations of masseter mRNA were amplified, corresponding to two different portions of the muscle, known to contain different amounts of myosin α heavy chain protein [1]). (A) In the presence (+) and absence (−) of reverse transcriptase. (B) In the presence (+) of reverse transcriptase. M, A, D, and S correspond to masseter, atria, diaphragm, and soleus muscles, respectively. Molecular weight markers (1,353, 1,078, 872, 603, 310, 281–271 bp) are indicated beside each figure.

strated that mRNA, and not genomic DNA, was amplified. No amplification could be detected with RNA from soleus, diaphragm (Fig. 1B), and EDL (not shown), which contain β and fast-type heavy chains.

The nucleotide sequences of 3 subclones of each masseter and atrial cDNA were determined. The sequence of one of the masseter cDNA subclones is presented in Fig. 2. It differed at one position from the sequence of atrial cDNA (the three atrial subclones were identical): a T instead of a C at position 1,862. This substitution was conservative and did not introduce an amino acid change. It also contained one single silent difference to the sequence of rabbit myosin α heavy chain previously published [6]: an A instead of a G at position 1,739. These results clearly indicate that the masseter contains a myosin α heavy chain, which confirms previous studies at the protein level [1,2].

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1260 GGG CCC ATC AAA GAG TCC CTG GAG AAG TCG GAG
CCC CGC CGC AAG GAG CTG GAG GAG AAG ATG GTG TCG
CTG CTG CAG GAG AAG AAT GAC CTG CAG CTC CAA GTG
CAG GCG GAA CAA GAC AAC CTC AAT GAT GCC GAG GAG
CGC TGC GAC CAG CTG ATC AAG AAC AAG ATC CAG CTG
GAG GCC AAG GTG AAG GAG ATG AAC GAG AGG CTG GAG
GAC GAG GAG GAG ATG AAC GCC GAG CTC ACC GCC AAG
AAG CGC AAG CTG GAA GAC GAG TGC TCC GAG CTC AAG
AAG GAC ATT GAC GAC CTG GAG CTG ACG CTG GCC AAG
GTG GAG AAG GAG AAG CAC GCA ACC GAG AAC AAG GTG
AAG AAC CTG ACA GAG GAG ATG GCT GGG CTG GAC GAG
ATC ATC GCC AAG CTC ACC AAG GAG AAG AAA GCT CTG
CAA GAG GCC CAC CAG CAG GCC CTA GAT GAC CTT CAG
GCT GAG GAG GAC AAA GTC AAT ACT CTG ACC AAG GCC
AAG CTC AAG CTG GAG CAG CAG GTG GAC GAT CTG GAG
GGA TCC CTG GAG CAG GAG AAG AAG GTG CGC ATG GAC
CTG GAG CGA GCC AAG CGG AAG CTG GAG GGT GAC CTG
AAG CTG ACC CAG GAG AGC ATC ATG GAC CTG GAG AAT
GAC AAG CTG CAG CTG GAG GAG AGG CTC AAG AAG AAG
GAG TTT GAC ATC AGT CAG CTG 1961
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Fig 2. Nucleotide sequence of most of the S2 subfragment of masseter myosin α heavy chain cDNA, numbered as in [6]; this cDNA fragment corresponds to nucleotides 2,674–3,375 of the myosin heavy chain sequence published in [10]. (+) designates the single difference with the atrial myosin sequence determined in this work. (●) designates the single difference observed between the masseter and atrial myosin sequences determined in the present study and the published sequence of myosin α heavy chain [6]. (▲) designates the two differences with the sequence of the third masseter myosin heavy chain subclone.

The amino acid sequence of the masseter myosin α heavy chain is presented in Fig. 3, together with the sequences of β and fast-type myosin heavy chains [6]; their comparison emphasizes the differences between

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α M4 2 0 G R I K E S L E K S E A R R K E L E E K M V S L L Q
β       A - V - - A - - - - - - - - - - - - - T - - - - -
F       E K T - - - - A - A - - K - - - - - - - - A - M -

E K N D L Q L Q V Q A E Q D N L N D A E E R C D Q L I K N K I
- - - - - - - - - - - - - - - - - - A - - - - - - - - - - -
- - - - - - - - - - - - - A - S - A - - - - - Q - - - - T - -

Q L E A K V K E M N E R L E D E E E M N A E L T A K K R K L E
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
- - - - - I - - V T - - A - - - - - I - - - - - - - - - - -

D E C S E L K K D I D D L E L T L A K V E K E K H A T E N K V
- - - - - - - R - - - - - - - - - - - - - - - - - - - - - -
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - -

K N L T E E M A G L D E I I A K L T K E K K A L Q E A H Q Q A
- - - - - - - - - - - - - - - - - - - - K - - - - - - - - - -
- - - - - - - - - - - - N - - - - - - - - - - - - - - - - T

L D D L Q A E E D K V N T L T K A K L K L E Q Q V D D L E G S
- - - - - - - - - - - - - - - - - - - V - - - - - - - - - -
- - - - - - - - - - - - - - - - - - T - - A - - - - - - - - -

L E Q E K K V R M D L E R A K R K L E G D L K L T Q E S I M D
- - - - - - - - - - - - - - - - - - - - - - - - - - - - - -
- - - - - - I - - - - - - - - - - - - - - - - I L - - - T - -

L E N D K L Q L E E R L K K K E F D I S Q L 6 5 3
- - - - - Q - - D - - - - - - D - E L N A -
I - - - - Q - - D - K - - - - - - E M T N -
```

Fig. 3. Amino acid sequence αM of most of the S2 subfragment of masseter myosin α heavy chain numbered as in [6]; this protein fragment corresponds to amino acids 859–1,092 of the myosin heavy chain sequence published in [10]. Comparison with β and fast-type F myosin heavy chains [6]; only the amino acids which differ from those in the masseter myosin α heavy chain are indicated.

the three sequences in this subfragment S2 region of the myosin heavy chain.

The sequence of the second masseter cDNA subclone was the same as that presented in Fig. 2. The sequence of a third subclone contained two substitutions: a G instead of a C at position 1,824, introducing the replacement of an arginine by a glycine, and a C instead of a T at position 1,888, introducing the replacement of an isoleucine by a threonine. It is possible that these substitutions were artefacts created by the reverse transcriptase or Taq polymerase. However, previous studies have suggested that there may be more than one variant of the myosin α heavy chain [8–10]. We may therefore have detected the mRNAs coding for two different variants.

From this work, we may conclude that the expression of the myosin α heavy chain gene is not restricted to cardiac muscle: it is also expressed in rabbit masticatory muscles. We showed recently that the regulation by thyroid and androgenic hormones of the V1 isoform, composed of α heavy chains and of slow or ventricular light chains, was opposite in the masticatory muscles and in the cardiac muscle [11]. The myosin α heavy chain may therefore be differently regulated in different muscles [12]. Further work is needed to characterize the regulation of the expression of a myosin α heavy chain in masticatory muscles.

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